the peptidic-aggregates. The addition of each osmolyte or a mixture of both increases the conformational stability of the six peptide amyloid-prone aggregate. All the studied osmolytes, decrease the number of peptide-water hydrogen bonds, increase the peptide-osmolyte hydrogen bonds, while the number of the intrapeptide hydrogen bonds remains almost unchanged. This result is in good agreement with the suggested mechanism of osmolytes replacing water molecules on the peptide aggregate surface. In contrast our molecular dynamics simulation has uncovered that the conformational stability of the 12 peptide aggregate was very similar in water, in presence of each osmolyte or a mixture of both. This effect is most probably due to the higher content of intrapeptide hydrogen bonds existing in the hydrophobic core formed within the 12 peptide aggregate.

Protein Conformation II

2252-Pos Board B22

Conformational Dynamics of the *E. Coli* SOS Mutagenesis Protein UmuD

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The SOS response in *Escherichia coli* involves the induction of at least 57 genes in response to DNA damage, including the *umuD* gene. $UmuD_2$ is a homodimer of 139-amino acid subunits that interacts with RecA/ssDNA nucleoprotein filaments, resulting in cleavage of its N-terminal 24-amino acids to yield $UmuD'_2$. The full-length form UmuD plays a role in a primitive DNA damage checkpoint and prevents mutagenesis, whereas the cleaved form facilitates mutagenesis by Y family DNA polymerase UmuC. The goal of our research is to determine the conformation and dynamics of UmuD in order to understand its regulatory role in response to DNA damage. $UmuD_2$ and $UmuD'_2$ are both exceptionally tight dimers, with picoMolar dissociation constants. However, we find that a monomeric variant of UmuD maintains essentially all biological roles of $UmuD_2$. This surprising finding suggests that UmuD can cleave in either a *cis* or *trans* conformation. By forming alternate dimeric forms of UmuD, we determined that cleavage in *trans* is more efficient than cleavage in *cis*. $UmuD_2$ and $UmuD'_2$ readily exchange monomers to form the heterodimer UmuDD', which is the most stable dimeric form. We are investigating the kinetics and mechanism of exchange of UmuD dimers and the UmuDD' heterodimer by using FRET assays and native PAGE. The different dimeric forms of the *umuD* gene products exchange on the minute time scale, and each dimer can continue to exchange once formed. Designed site-directed mutations are expected to allow us to determine the mechanism of UmuD dimer exchange.

2253-Pos Board B23

Persistent α-Helical Content and Local Helical Structural Fluctuations from a Molten Globule to Ordered Peptide Transition

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Structural information about proteins can provide valuable insight into folding mechanisms and dynamics, giving us information about protein function and interactions within biological systems. Upon interaction with various moieties, proteins have the potential to undergo a variety of conformational changes. Extensive studies have been done using deep UV resonance Raman (dUVRR) spectroscopy to study the secondary structure of proteins. Here, we present the results of our experiments, where we simultaneously monitored both secondary and tertiary structure of de novo synthesized protein, HP7. HP7 is a four-helix bundle that retains its secondary structure while altering its tertiary structure dependent upon the attachment of a heme group. In studying the apo- and holo-proteins, we determine that dUVRR spectroscopy is a promising tool for the simultaneous study of both protein secondary and tertiary structure.

2254-Pos Board B24

Probing the Temperature Dependence of Structure and Dynamics of Thermophilic Lactate Dehydrogenase from Th. Maritima

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Thermophilic lactate dehydrogenase (LDH) from Thermotoga maritima demonstrates a drastic increase in Km with increased temperature, compared with that of mesophilic LDH. To investigate such difference and the temperature adaptation of proteins, FTIR is used to probe the structure dependence on the temperature by monitoring amide-I band and CO stretch of oxamate in the ternary complex. It has been found that, although protein denaturing are not observed with an increased temperature up to 80 C (the temperature for optimum growth of Th. maritima), the protein structure as reported by T. Dams et al., shows difference between room temperature and 80 C. FRET and laser induced temperature jump techniques are employed to study the dynamics of ligand-protein complexes of TmLDH on the nanosecond time scale.

2255-Pos Board B25

Heterospectral 2D Correlation Analysis of the Redox Induced Two-Step Conformational Transition of Cytochrome C

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Redox proteins are the main components of the respiratory chain. Even though their structure is known in great detail, information about structure-function relationships is still sparse. Vibrational spectroscopy is well designed to fill this gap Raman- and Infrared-Spectroscopy yielding complementary information, they are usually applied separately. Information about heme centres and peptide structures are thus obtained, which are Raman and infrared-active, respectively. If, for example the protein is immobilized on an electrode, changes of the protein-backbone and the heme-centre may be observed as a function of an applied potential. To fully understand the mechanism of the interaction of heme-centre with the protein-backbone information obtained by both techniques have to be correlated.

Cytochrome c (cc) containing a Raman-active redox-centre, surrounded by the infrared-active backbone, has been used as a benchmark system to introduce the concept of hetero 2D correlation spectroscopy.

The redox transition of the heme group as a function of potential could be correlated with conformational changes of the following peptide groups 32-40, whereas other groups such a 14-19 and 57-59 do not change simultaneously. Hence we can conclude that changes in the protein backbone as a function of the redox transition occur in a two-step process. This is consistent with MD calculations showing that the a-helical structure is elongated during oxidation whereas a β-structure is formed.

2256-Pos Board B26

Investigations of the ATPase Mechanism of HSP70 Molecular Chaperones upon Substrate Binding using Protein Engineering and Computational Techniques

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Hsp70 chaperones play mportant roles in cells including protein folding, trafficking, degradation and enabling survival under stress conditions. DnaK is an *Echerichia coli* Hsp70 homolog comprising a 44 kDa ATPase domain (NBD) and a 25 kDa substrate-binding domain (SBD). DnaK has two nucleotide substrate-affinity states: In the ATP-bound low substrate-affinity state, substrate binding and release occur rapidly, whereas in the ADP-bound high substrate-affinity state, slower substrate binding and release kinetics are observed. Communication between the two domains is essential for chaperone function and is mediated via a conserved hydrophobic linker region (^{384}GDVKDVLLL^{392}). Previous studies showed that when this flexible linker interacts with the ATPase domain, which was studied by the construct containing the entire linker, DnaK(1-392), an enhanced ATPase rate is observed compared to the construct lacking the conserved ^{389}VLLL^{392} linker region, DnaK(1-388). This observation suggests that structural rearrangements caused by linker docking adopt the ATPase domain in a closed conformation, leading to an enhanced, pH-dependent ATPase activity. Here, our aim is to delineate the residues that are responsible for the linker induced conformational rearrangements. In that line, using molecular dynamic simulations we identified two sets of amino acids at the lobe interface of the ATPase domain that might be critical in the stabilization of the domain in the so called ''open'' and ''closed'' conformations. We made point mutations for these sites on both DnaK(1-392) and DnaK(1-388) constructs, and studied the structural and functional effects of these residues on the ATPase domain using pH varied stability measurements by circular dichroism and activity measurements as a function of pH, respectively. Our experimental results also point to the significance of these residues in the domain rearrangements when triggered by linker binding.

2257-Pos Board B27

Dynamic Motions of the G Protein Subunit G Alpha I1 While Complexed to the GEF Ric-8A

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Canonical G-protein signaling pathways are activated when agonist-bound G protein coupled receptors (GPCR), acting as guanine nucleotide exchange factors (GEF), promote the exchange of GDP for GTP on Gα subunits present in GαGDP:Gβγ heterotrimers. GEFs catalyze product release and substrate